Localized Surface Plasmon Coupled Fluorescence Fiber-optic Biosensor for Severe Acute Respiratory Syndrome Coronavirus Nucleocapsid Protein Detection

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Abstract

Detection of severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in human serum using localized surface plasmon coupled fluorescence (LSPCF) fiber-optic biosensor. The detection limit at 1pg/mL in human serum is successfully demonstrated.

Introduction

Severe acute respiratory syndrome (SARS) is a highly infectious interstitial pneumonia that causes death in a great portion of patients [1]. SARS is caused by the coronavirus (SARS-CoV) that radiates in patients' respiratory secretions after infection [1, 2]. In 2003, the outbreak of SARS in the Southeast Asia and other continents affected over 30 countries [3, 4]. This disease is highly contagious and has the potential to cause a very large scale epidemic again in the absence of a vaccine or effective therapeutic drugs [3, 5-7]. The key to prevent and control future outbreak of SARS is to block transmissions of infection through a strict quarantine policy [3]. Therefore, a rapid, sensitive, specific, and accurate diagnostic method is essential for the assessment of patients identified [8, 3]. Currently, there are several diagnostic options for the detection of SARS such as detecting SARS-CoV antigens such as nucleocapsid (N) protein [8, 9].

From properties of localized surface plasmon coupled fluorescence (LSPCF), a novel fiber-optic biosensor was proposed by our group on protein-protein interactions [10, 11]. In this experiment, a highly sensitive LSPCF fiber-optic biosensor is used to rapidly detect SARS-CoV N protein in dilute serum (d-serum) for early detection of SARS-CoV infection.

Experiment Section

Materials:

Capture antibody, target antigen and secondary antibody were prepared by professor Yi-Ming Chen (Institute of Microbiology and Immunology, National Yang-Ming University, Taipei, Taiwan). Human serums were prepared by Professor Jason C. Huang (Department of Biotechnology and Laboratory Science in Medicine, National Yang-Ming University, Taipei, Taiwan).

Surface modification of the PMMA fiber:

In this experiment, the decladding of polymethyl methacrylate (PMMA) fiber was done by immersing the fiber in ethyl acetate. Subsequently, the optical fiber's decladding portion was washed with 2-propanol to clean the surface. A chemical adsorption method was used by covalent binding force in order to immobilize the capture antibody on the surface of decladding portion. The protocol of chemical adsorption was described previously [12].

Biorecognition molecules and sandwich immunoassay:

The fiber-optic biosensor is constructed based on sandwiched biomolecular complex, < capture antibody / target antigen / secondary-antibody (fluorescence probe) >. The capture antibody is the mouse monoclonal anti-SARS-CoV N protein (anti-N protein I). In order to avoid the non-specific binding of fluorescence probe on the modified decladding portion during measurement, fibers are immersed in BSA solution (10mg/mL) before incubating with target antigens. The target antigen (SARS-CoV N protein) in PBS solution or d-serum was injected into the reaction chamber to interact with the immobilized capture antibody to form the < anti-N protein I / SARS-CoV N protein > complex.

Preparation of the fluorescence probe:

To produce the fluorescence probe, Au-PA with a concentration of 9×10^{9}particles/mL is mixed with the fluorophore to label another mouse monoclonal anti-SARS-CoV N protein (anti-N protein II). In this experiment, the concentration of fluorophore labeled anti-N protein II was 100ng/mL.

Measurement:

This fiber-optic biosensor combines LSPCF technique with sandwich immunoassay. The sandwich structure is built on the modified decladding portion of the PMMA fiber. LSPs which are excited by evanescent wave produce significant enhancement of the localized electromagnetic field near GNP surface. Thereafter the fluorophores labeled secondary-antibodies are excited by enhanced field of LSPs.

Results

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The linear relationship between the fluorescence signal and SARS-CoV N protein concentration (in logarithmic scale) over the range of 0.1pg/mL to 1ng/mL in PBS solution is shown clearly in Figure 1. Figure 2 shows the linear relationship between the fluorescence signal and different concentrations of SARS-CoV N protein in d-serum over the same range of 0.1pg/mL to 1ng/mL. Both figures also exhibit linearity with the strength of fluorescent signal. The correlation coefficients of the two experiments are 0.9469 and 0.9624, respectively.

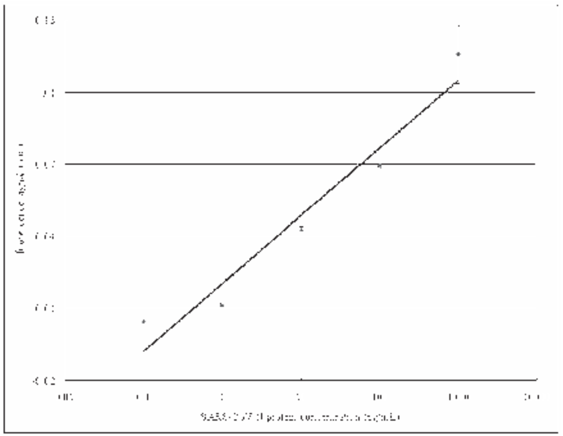

Fig. 1. [$y = 0.0122Ln(x) + 0.0206$; $R^2 = 0.9469$] SARS-CoV N protein concentration (in PBS solution) versus fluorescent signal.

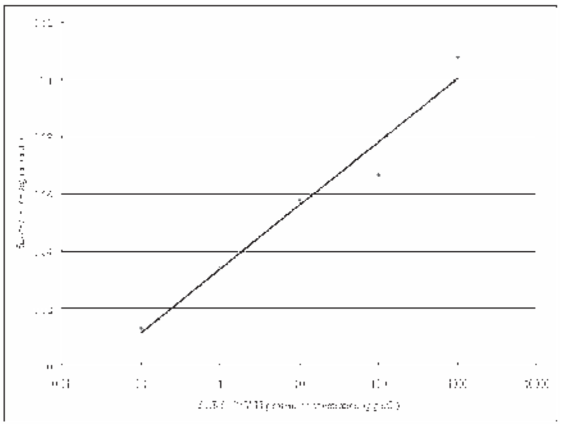

Fig. 2. [$y = 0.0096Ln(x) + 0.0337$; $R^2 = 0.9624$] SARS-CoV N protein concentration (in d-serum) versus fluorescent signal. The error bar of the measurement is the same size of dot in this figure.

Conclusions

We successfully demonstrated LSPCF fiber-optical biosensor on SARS-CoV N protein in human serum is suitable for clinical diagnosis to identify SARS patients efficiently. LSPCF fiber-optic biosensor has a simple setup, is easy to operate and also performs a detection limit reaching to 0.1pg/ml in d-serum.

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